Persistence of neostigmine-like activity in tissues of the rat after prolonged oral administration

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Tolerance to neostigmine occurs in rats after prolonged oral administration of this drug (Buckley & Heading, 1970), and tolerance to neostigmine persists for up to 3 weeks after the plasma cholinesterase (ChE) activity attains values within the range of non-treated control rats. These results suggest that tolerance to neostigmine is not directly linked with inhibition of plasma cholinesterase. Further experiments were designed to determine the relationship between cholinesterase inhibition in plasma and selected neural tissue (ganglion). The term 'cholinesterase' is used collectively in this report.

Eighteen rats were used, six control (non-treated) and twelve which received neostigmine bromide in their drinking water at an initial dose level of 0·33 (mmol/kg)/day. The dose was increased gradually for 6 weeks until the dose was 0·59 (mmol/kg)/day, when treatment was stopped. The ChE activities of plasma and stellate ganglion of six treated rats were measured 3 weeks after treatment ceased and similarly of six rats 5 weeks after cessation of treatment. Plasma cholinesterase was measured by the method of Ellman, Courtney, Andres & Featherstone (1961) and ganglion cholinesterase by the method of Potter (1967).

Three weeks after neostigmine treatment there was no significant difference between the levels of plasma cholinesterase from treated and control rats. However, when the anticholinesterase activity of plasma from these treated rats was investigated by mixing with an equal volume of plasma from a control rat, it was observed that plasma from three of them had pronounced anticholinesterase activity. Five weeks after the end of neostigmine treatment there was no difference between levels of plasma cholinesterase from treated and control rats and there was no evidence of anticholinesterase activity in the plasma.

The presence of carbamylated ChE in homogenates of stellate ganglion was detected by assaying cholinesterase 30 min after preparation and again 24 h later. This incubation of tissue in pH 7·4 buffer without substrate allowed time for decarbamylation of cholinesterase to occur. The ratio of cholinesterase activity after 24 h expressed as a percentage of the cholinesterase activity after 30 min was called 'decarbamylation ratio' and was used to indicate the presence or absence of inhibition in the tissue.

The mean decarbamylation ratios of stellate ganglia preparations removed 3 weeks and 5 weeks after cessation of treatment were significantly higher than the corresponding controls ($P < 0.05$). This was taken as evidence of the presence of inhibited enzyme.

It is concluded from these experiments that after prolonged neostigmine treatment: (1) plasma cholinesterase activity can apparently be within the range of control values although the plasma may contain substance(s) with anticholinesterase activity, and (2) even when plasma is apparently free from anticholinesterase activity, inhibition of cholinesterase in ganglion tissue persists, possibly due to the presence of neostigmine or its metabolites.

The work was financed by a grant from the M.R.C.

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Effects of phenobarbitone and leptazol on rat brain lysosomes

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Phenobarbitone influences the membrane function of erythrocytes (Coldman & Good, 1969), cerebral cortex slices (Gilbert, Ortiz & Millichap, 1966) and synaptosomes (Balfour & Gilbert, 1971), but effects on cerebral lysosomal membranes have received little attention. Many drugs stabilize or labilize lysosomes (Weissman, 1968) and it is possible that the effects of phenobarbitone on lysosomes of the central nervous system might be involved in the mechanism of its anticonvulsant effect. The effects of phenobarbitone on release of acid phosphatase from lysosomes of the rat cerebral cortex have been determined: (i) after injecting phenobarbitone into rats and subsequently incubating the mitochondrial fraction in 0·25 M sucrose solution and (ii) after incubating the mitochondrial fraction from untreated animals in media containing phenobarbitone.

Male Sprague-Dawley rats were injected intraperitoneally with phenobarbitone sodium, thiopentone sodium or with 0·9% NaCl solution, 1 h before decapitation. The mitochondrial fraction containing lysosomes, was prepared as described by Koenig, Gaines, McDonald, Gray & Scott (1964). It was incubated for 30 min in 0·25 M sucrose and released acid phosphatase activity was determined by the method of Gianetto & de Duve (1955). The activity of the enzyme released was calculated as a percentage of the total activity released by 0·01% Triton X-100.

In preliminary experiments, when an anaesthetic dose (150 mg/kg) of phenobarbitone sodium was used, the free acid phosphatase activity was reduced by 14% compared to controls ($P<0·01$), whereas in rats anaesthetized with thiopentone sodium (40 mg/kg), the free activity was reduced by 8% and this was not significant at the 5% level. When rats were injected with a smaller dose of phenobarbitone sodium (50 mg/kg) daily for 4 days, they did not appear to be sedated at the time of killing (18 h after the last injection) and the free acid phosphatase activity was reduced by 9% ($P<0·05$). When rats were injected with leptazol (64 mg/kg) and decapitated during a convulsion, the free acid phosphatase activity was increased by 18% ($P<0·01$).

In other experiments, the mitochondrial fraction obtained from cerebral cortex was incubated for 30 min in 0·17 M sucrose containing 83 mM tris-maleate buffer pH 7·4, with or without phenobarbitone (0·01, 0·1, 2·0 or 5·0 mM) and the medium was then made hypotonic by the addition of water (2 vol. to 3 vol. medium) and the incubation was continued for 30 minutes. The release of acid phosphatase activity by osmotic shock was decreased by 2·0 mM (by 61%) and 5·0 mM (by 66%) phenobarbitone sodium.

These results suggest that phenobarbitone stabilizes brain lysosomes to a significant extent.

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